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PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Gary Ruvkun et al.
Serial No.: 09/844,353
Filed : April 27, 2001
Title : THERAPEUTIC AND DIAGNOSTIC TOOLS FOR IMPAIRED GLUCOSE TOLERANCE CONDITIONS

Art Unit: 1636
Examiner: Sumesh Kaushal

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ORIGINAL

DECLARATION OF DR. GARY RUVKUN

1. I am an inventor on the above-captioned patent application.

2. I have read the Office Action that was mailed on April 24, 2003.

3. Although *C. elegans* and humans are evolutionarily distant organisms, human AFX, FKHRL1, FKHR and *C. elegans daf-16* proteins are highly related. In fact, we have found that DAF-16 and human FKHRL1 are so closely related that the human protein is able to functionally substitute for *C. elegans* DAF-16 *in vivo*.

4. We have shown (Lee et al. Curr. Biol. 11:1950-1957, 2001, Exhibit A) that when a *daf-16* human homolog, FKHRL1, was expressed under the control of the *daf16β* promoter in worms having mutations in *daf-16* and *daf-2*, the human protein was able to replace the worm DAF-16 protein, although the human protein's ability to rescue the *daf-*

16 phenotype (70%) was somewhat weaker than that of a *C. elegans* DAF-16 protein (100%). These results prove that the human and *C. elegans* proteins are orthologs. Other highly similar DAF-16 family members would also be expected to substitute for *C. elegans* DAF-16.

5. In collaboration with Nargis Nasrin and Maria Alexander-Bridges, we have also shown that *C. elegans* DAF-16 functions similarly to human DAF-16 homologs when the *C. elegans* protein is expressed in cultured human hepatocellular carcinoma cells (HepG2 cells), as evidenced in Exhibit B (Nasrin et al., PNAS 97:10412-10417, 2000, Exhibit B). In HepG2 cells, DAF-16 and its mammalian homologs, FKHR, FKHRL1, and AFX, activated transcription through the insulin growth factor binding protein (IGFBP)-1-insulin responsive element (IRE). We also found that *C. elegans* DAF-16 and FKHR interacted with both the KIX and E1A/SRC interaction domains of p300/Creb-binding protein (CBP), as well as the steroid receptor coactivator (SRC). We concluded that DAF-16 and FKHR act as accessory factors to the glucocorticoid response, by recruiting the p300/CBP /SRC coactivator complex to a forkhead factor site in the IGFBP-1 promoter, which allowed the cells to integrate the effects of glucocorticoids and insulin on genes that carry this site. Given this result, it is fully expected that other highly related DAF-16 proteins would function similarly.

6. Transgenic nematodes are inherently different from transgenic mammals. Producing a transgenic nematode is a routine matter, requiring no more than standard methods. Transgenic *C. elegans* are made by microinjecting plasmid or linear DNA with a selectable marker. *In vivo*, the DNA is assembled into concatamers that are properly

regulated. Transgenic nematodes are used routinely to identify a coding region that corresponds to a genetically-defined locus, and to rescue mutations by complementation. In nematodes, transgene expression is reliably used to produce a wild-type phenotype. Moreover, all experiments are routinely carried out in isogenic strains.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 7/22/03

Gary Ruvkun, Ph.D.